*Sribharathy Vijayagopal [1],Viswadhara Nagarajan[1], Jeyaprabha Chellappa[2]**

[1]*Department of chemistry, Anna Adarsh College for Women, Anna Nagar, Chennai , India,* [2]*Department of Science and Humanities – Chemistry, Anna University – University College of Engineering Dindigul, Dindigul , India*

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Bio-inspired synthesis of sulphur nanoparticles and its application

ABSTRACT

We employed an economical, swift, and environmentally friendly approach to synthesize sulphur nanoparticles, utilizing an extract from Ficus religiosa leaves. Comprehensive characterization of these nanoparticles was performed through UV-Visible Spectroscopy, FTIR, XRD, and FESEM techniques. The X-Ray diffraction method unequivocally confirmed the nanometre-scale dimensions of the synthesized sulphur nanoparticles. SEM analysis elucidated their spherical morphology, while XRD data indicated a crystalline size of 23.4 nm for these prepared nanoparticles. Besides, the antibacterial assessment of sulphur nanoparticles produced from Ficus religiosa leaves exhibited superior bioactivity against harmful bacteria species such as Escherichia Coli and Staphylococcus aureus.

Keywords: *Sulphur nanoparticles, Ficus religiosa, bioactivity, green synthesis*

1. INTRODUCTION

Sulphur ranks as the third most abundant mineral in the human body, found within essential amino acids like methionine and cysteine, crucial for protein synthesis in tissues such as skin, hair, and nails. Our body acquires necessary Sulphur from diverse sources, including animal and plant proteins, sulfinates, allicin, and sulphides. Additionally, Sulphur is a component of thiamine (vitamin B-1) & biotin (vitamin H) [1, 2].Sulphur plays vital roles in DNA construction, cell protection against diseases like cancer, metabolism, and the health of skin, tendons, and ligaments. Methionine, an essential amino acid, must be sourced from proteins, while cysteine, a non-essential amino acid, is synthesized within the body.

Furthermore, Sulphur is present in supplements like glucosamine sulfate, chondroitin sulfate, and methylsulfonylmethane (MSM), often used for alleviating joint pain and inflammation [3]. Some natural health practitioners speculate on their potential to enhance skin, fingernails, and other tissues, possibly due to the presence of serum sulphates.

However, these therapeutic claims lack comprehensive scientific validation.It's imperative to consult healthcare professionals and rely on evidence-based research before using any supplements for therapeutic purposes. While Sulphur is undeniably vital for bodily functions, its specific impact on skin, nails, and tissues may vary from person to person, necessitating further research for conclusive evidence.

Glucosamine sulphate, chondroitin sulphate, and methylsulfonylmethane (MSM) are commonly used supplements for joint health, and they do contain Sulphur in various forms. Some individuals believe these supplements can also benefit the skin and other tissues, but these claims are often anecdotal and not supported by rigorous scientific studies.It's crucial to consult with healthcare professionals and rely on evidence-based research before using any supplements for therapeutic purposes. While Sulphur is undoubtedly important for the body's functions, its specific effects on skin, nails, and other tissues may vary from person to person and require further research to establish definitive conclusions.

However, its application in agrochemical industries is currently limited due to the requirement of bulk quantities and the potential development of resistance in target species. Additionally, the high hydrophobicity of Sulphur has restricted its practical use. Nevertheless, the emergence of nanotechnology offers a new avenue

*Corresponding author: Jeyaprabha Chellappa
E-mail: drcjeyaprabha@gmail.com
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for Sulphur utilization. Surface-modified nano-sized Sulphur nanoparticles (SNPs) have shown remarkable antimicrobial and antifungal activities, as reported in previous studies [4-6]. Polymeric sulphur nanoparticles are found to be effective against gram-positive methicillin resistantS.aureus and gram-negative P.aeruginosa [7,8]. These SNPs have also been utilized in the production of anticancer and antibacterial agents [9].

GREEN SYNTHESIS OF NANOMATERIALs

In the past decade, nanoscience and technology have seen intriguing developments in novel synthesis methods for various nanomaterials, including metal nanoparticles; quantum dots, carbon nanotubes, graphene materials and their related composites [10-15]. Researchers have explored two fundamental principles of synthesis, namely top-down and bottom-up methods, to achieve nanomaterials with desired sizes, shapes, and functionalities, as depicted in Fig.1[16]. Earlier techniques involved lithographic methods, ball milling, etching, and sputtering [17]. In the bottom-up approach, the nanoparticles are prepared from small molecules using techniques such as PVD (physical vapour deposition), CVD (chemical vapour deposition), sol-gel method, spray and laser pyrolysis methods, etc.The morphological characteristics of nanoparticles such as size, shape can be controlled by adjusting chemical concentrations and reaction conditions, such as temperature and pH.

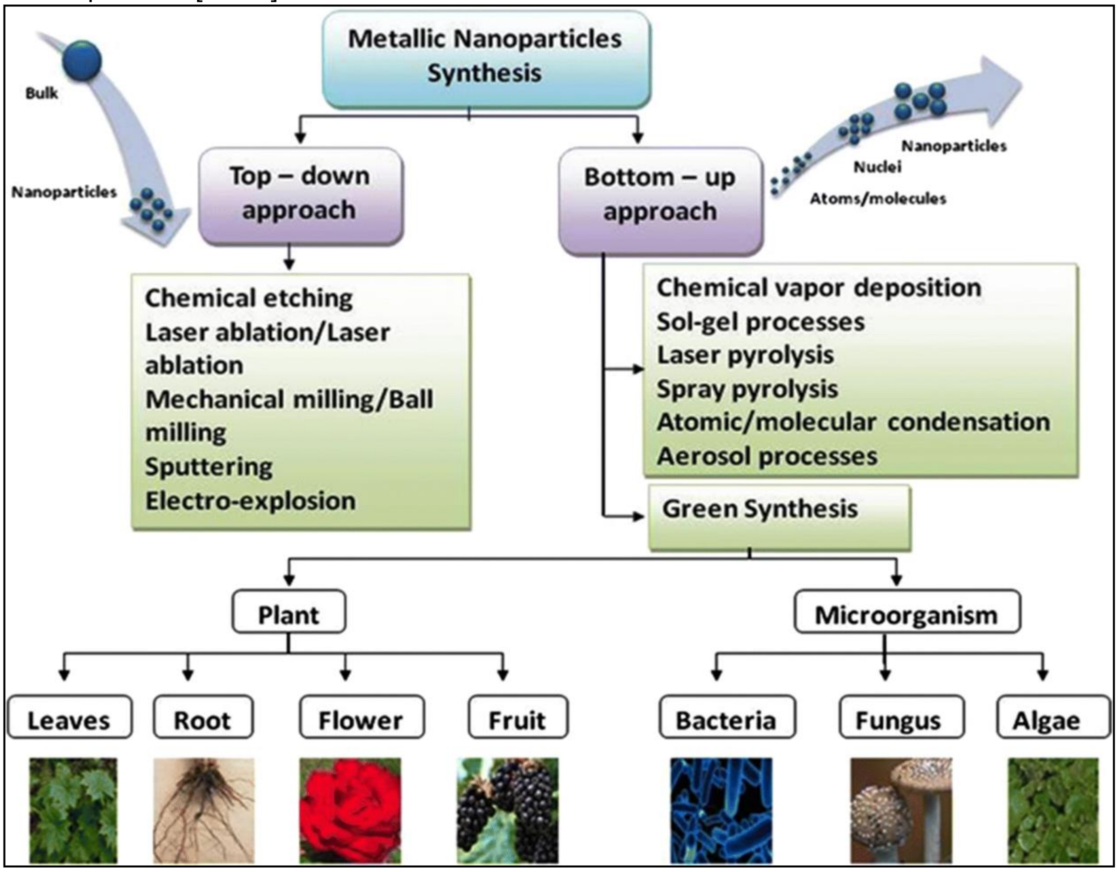

Figure 1. Top Down and Bottom-up approaches of synthesis of Nano particles [15]

However, these produced nanomaterials face challenges when applied practically including stability in aggressive environments, lack of understanding in fundamental mechanisms, modelling factors, bioaccumulation/toxicity concerns, extensive analysis requirements, need for skilful operators, challenges in device assembly and structures, and issues related to recycling/reuse/ regeneration. To address these limitations, there is a growing focus on green synthesis approaches in current materials science and technology research and development. Green synthesis methods, involving regulation, control, clean-up, and remediation processes, enhance the environmental friendliness of materials/ nanomaterials. Green synthesis principles include minimizing waste, reducing derivatives/pollution, and utilizing safer or non-toxic solvents/auxiliaries and renewable feedstock.

To prevent the formation of unwanted by-products and establish consistent, sustainable, eco-friendly synthesis procedures, the use of suitable solvent systems and natural resourcessuch as organic systems, is crucial. Green synthesis of metallic nanoparticles has gained momentum, especially utilizing biological materials viz., microorganisms – bacterial species, fungus species, algal extract and plant extracts. Among these methods, plant extract-mediated synthesis is a simple, easily adaptable and scalable process, making it easy to produce the nano scale materials on a large scale compared to microorganisms mediated synthesis. The resulting materials are collectively fall under the category of biogenic nanoparticles [18, 19].

'Green Synthesis' isvitaland mostly practiced method now-a-days, mainly to minimize the production of unwanted and harmful by-products formation during the build-up of reliable, sustainable and eco-friendly synthesis methods. The use of suitable solvent systems and naturally available resources are necessary to achieve the goal. Green synthesis of metallic nanoparticles has been accepted to have scope for various biological materials. Among the available eco-friendly methods of synthesis for nanoparticles, utilization of plant extracts is quite simple and easy process to prepare nanoparticles at large scale relative to other microorganisms mediated synthesis. These products are known collectively as biogenic nanoparticles [19, 20].

To synthesize sulphur nanoparticles, we utilized leaves from the *Ficus religiosa* tree, a significant and revered species in Indian culture due to its mythological, religious, and medicinal importance. Traditional Indian medicine has long utilized different parts of this tree for various ailments. The bark serves as an antibacterial, antiprotozoal, antiviral, astringent, and anti-diarrheal agent, treating conditions like gonorrhea and ulcers. Moreover, the leaves are used for skin diseases, exhibiting anti-venom properties and regulating menstrual cycles.

Both aqueous and ethanolic extracts from *F.religiosa*leaves demonstrated antibacterial effects against multiple strains, including Staphylococcus aureus, *Salmonella paratyphi, Shigella dysenteriae, Salmonella typhimurium, Pseudomonas aeruginosa, Bacillus subtillis, Escherichia coli,*etc. Notably, the methanolic extract of *F.religiosa*leaves inhibited the production of nitric oxide and proinflammatory cytokines in LPS-stimulated microglia via the mitogen-activated protein kinases (MAPK) pathway, as confirmed by cell viability, nitric oxide, and enzyme-linked immune-sorbent assays(ELISA)[21]. This extract showed robust anti-inflammatory properties in microglial activation, suggesting a potential neuroprotective effect against inflammation mediated by inflammatory mediators such as nitric oxide.

Synthesizing nanoparticles using plant extracts offers several advantages compared with other green synthesis methods because plants are eco-friendly and easy to handle [22]. Moreover, it offers energy efficiency, low toxicity, high yield, time-, cost-effectiveness, availability. Phytochemicals in plants, such as neo-clerodane flavanol glycosides, ergosterol, iridoid glycosides, phytoecdysones, other polyphenols, play an essential role in the green synthesis of nanoparticles as reducing, capping, and stabilizing agents [23].

In the present study, sulfur nanoparticles (SNPs) were prepared through an environmentally friendly process using *F.religiosa* leaf extract. *F.religiosa*leaves were selected due to their proven activity as an efficient biomaterial for the synthesis of nanoparticles and ease of availability.*F.religiosa* is tolerant to widely varying climatic conditions. *F.religiosa* is used in traditional medicine systems such as Ayurvedic, Siddha and Unani for disorders like asthma, diabetes, diarrhoea, gastric trouble, inflammatory disordersand infectiousdiseases.Moreover *F.religiosa* has a lifespan 900 and 1,500 years.

2. EXPERIMENTAL PROCEDURES

2.1. Materials required

*Ficus religiosa*leaves were collected from trees in Anna Nagar, Chennai. Sodium thiosulphate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$, 99.5%), citric acid ($C_6H_8O_7$) and deionized water were procured from lab chemicals (Chennai).

2.2. Preparation of leaf extract

Ficus religiosa leaves were washed thoroughly with tap water and then with deionized water until all the unwanted visible dirt particles were removed. Subsequently, the leaves were allowed to dry at room temperature to remove surface moisture. The dried leaves were chopped into fine pieces and 100gm of them were dispersed into 1000 ml of deionized water. The mixture was boiled at 100°C for 2 hours and filtered. The filtrate solution was used as a leaf extract. The supernatant liquid was found to exhibit fluorescence when kept under UV light and a laboratory test was performed to detect the presence of sulphur.

2.3.Preparation of sulphur nanoparticles (SNPS)

Before the synthesis of SNPs, all the glassware was cleaned using an *aqua regia* solution to remove the potential nucleation sites on the surface of the glassware. First, sodium thiosulpate pentahydrate (0.078 M) was dissolved in 250 ml deionised water and to this solution 100 ml *Ficus religiosa* leaf extract was added. After 5 min of continuous stirring, 125 ml of 20% citric acid ($C_6H_8O_7$) was added into the preceding reaction mixture. After the mixture was kept for 1 h at continues stirring, a precipitation was observed. The moisture was then centrifuged for 15 minutes at 5000 rpm and supernatant was removed. The precipitate was then washed and dried; the scheme diagram is shown **in Fig. 2.**

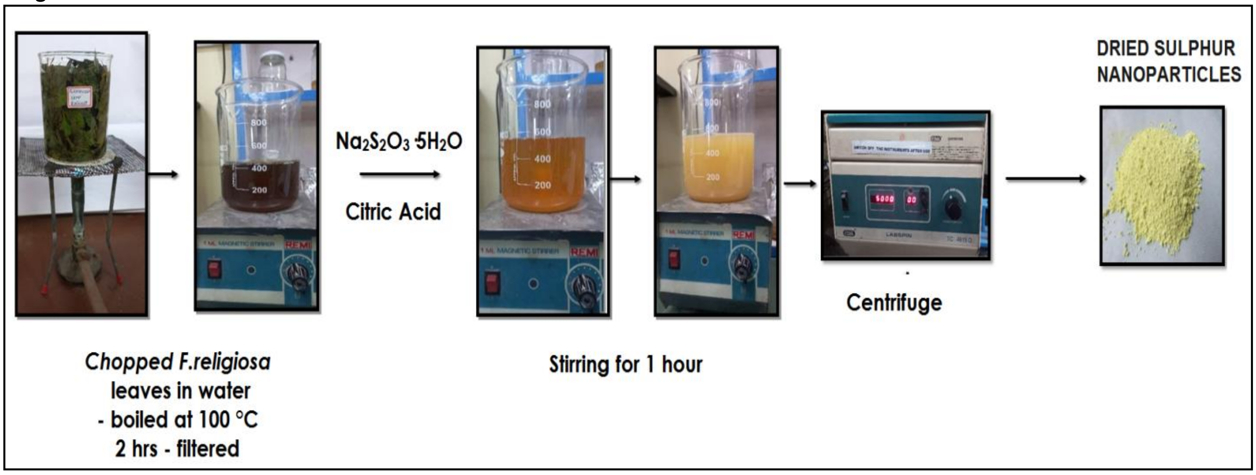

Figure 2. Scheme Diagram - Synthesis of Sulphur Nanoparticles

2.4. Characterization of sulphur nanoparticles (SNPS)

The prepared bio-inspired sulphur nano particles were characterized using various spectral techniques like UV-Visible, FTIR spectroscopy, XRD, SEM and EDAX. The UV-Visible studies were carried out using UV-Visible Spectrophoto-meter (Model - JAZ; Oceian Optics, USA), and FTIR spectral studies were carried out using Spectrum Two FT-IR / SP10 Software (Perkin Elmer).

The X-ray diffraction and SEM studies were carried out using P-XRD (2θ range5to 130) (Model-Smart Lab SE X-Ray; Rigaku, Japan and Model-Quattro S; Make-Thermo-Fisher Scientific, USA).

2.5. Antibacterial activity of SNPS

The efficiency of the antibacterial property of sulphur nanoparticles was examined by the disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured into the Petri plate. The activities of bio-inspired sulphur nanoparticles were evaluated individually and in combination with the antibiotics by soaking 20μl sulphur nanoparticles solution in the respective antibiotic discs and sulphur nanoparticles alone on a sterile disc. The leaf extract was maintained as a control. On incubation at 37°C for 24 h, the zones of inhibition were measured in millimeter.

3. RESULT AND DISCUSSION

3.1. Testing of sulphur by lassaigne's test

Sulphur present in the resulting product is detected by Lassaigne's test by taking a small piece of Na metal is heated in a fusion tube with the precipitate obtained. To the Lassaigne's filtrate, few drops of sodium nitroprusside have been added and the solution turned purple which confirms the presence of sulphur (Fig. 3).

Figure 3. Testing of Sulphur by Lassaigne's test

3.2. UV analysis of sulphur nanoparticles (SNPS)

Fig. 4 shows the UV-Vis spectrum of sulphur nanoparticles (SNPs), it is well known that α-sulphur exhibits an optical absorption maximum in the range of 260–280 nm.The peak at 274nm in figure 3 indicates the successful formation of SNPs. A secondary peak at ~324nm corresponds to the b2 → e3 transition [24].

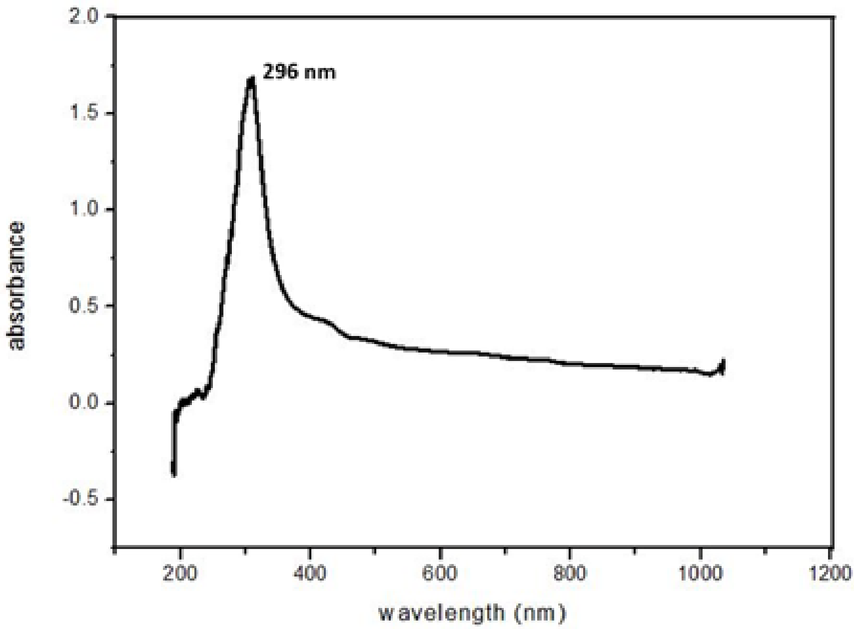

Figure 4. UV-Visible spectrum of sulphur nanoparticles

3.3. FTIR analysis of sulphur nanoparticles (SNPS)

Fig. 5 showsan FTIR spectrum and the corresponding absorbance peaks for SNPs. The prominent peaks at 3334 cm^{-1}, 2915 cm^{-1}, and 1393 cm^{-1} show the presence of –OH groups, –N-H stretching vibration, O=C=O bonds, –COO (sym. Stretching) and these bands revealed the presence of phytoconstituents of *F.religiosa* leaf extract. The peaks at 1584 cm^{-1}, 1082 cm^{-1}, and 880 cm^{-1} are attributed to the presence of –C=O (stretching), –CN bending vibration (secondary amide), O—S, and N—S stretching vibration of sulphur nanoparticles. The nominal changes in peak values, i.e.1052 cm^{-1}, 1513 cm^{-1}, 876 cm^{-1}, 656 cm^{-1}i.e. the peak shift arises due to the interaction of SNPs with amine groups in the biomolecules. The results indicate that the biomolecules from *F.religiosa* leaf extract proteins were bound onto the surface of as-prepared SNPs [25,26].

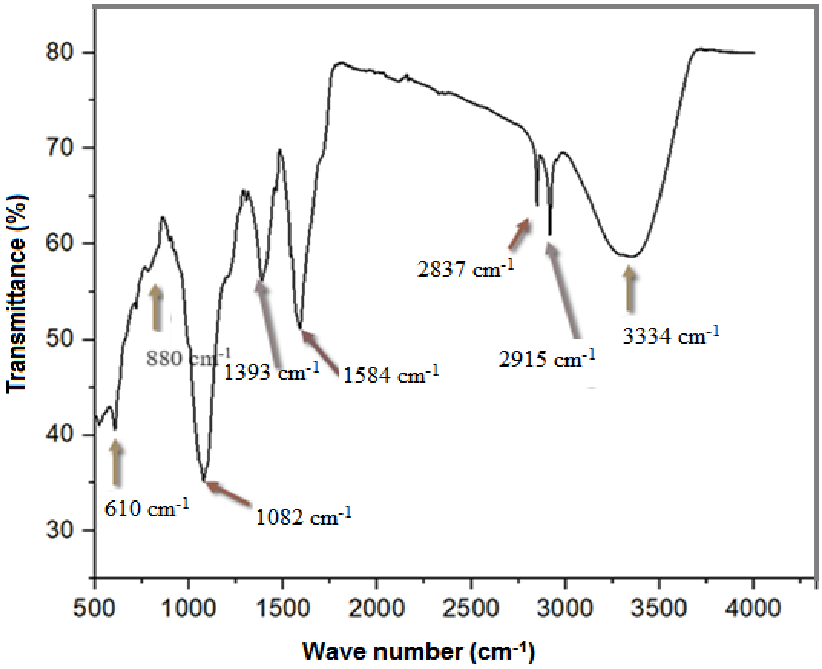

Figure 5. FTIR spectrum of sulphur nanoparticles

3.4.X-Ray diffraction analysis

Fig. 6shows a typical XRD pattern of SNPs. All the diffraction peaks are in good conformity with sulphur and no other impurity phases are observed, indicating the good phase purity of SNPs.

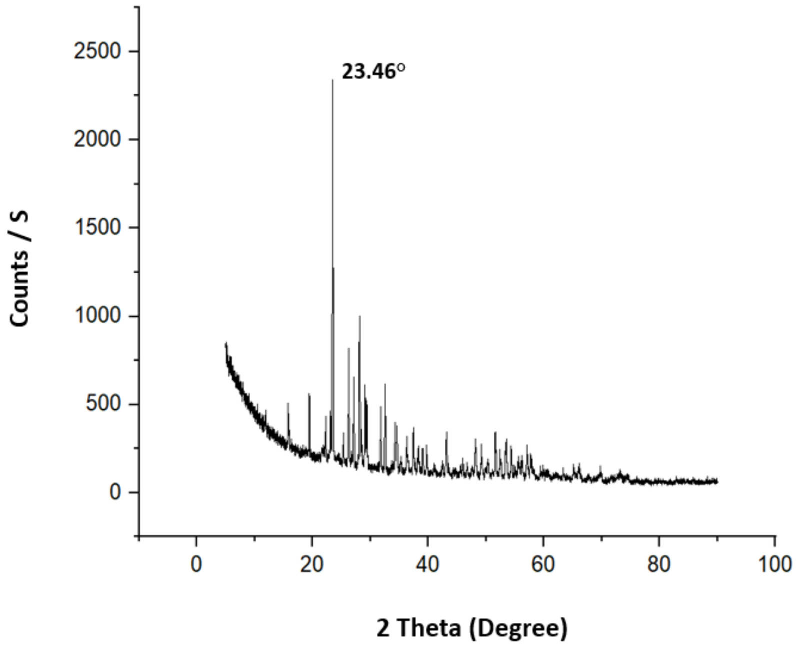

Figure 6. X-RD pattern of sulphur nanoparticles produced from F.religiosa leaf extract

The Debye-Scherrer formula was used to calculate the average crystalline size of the SNPs, which was found to be 23.4nm.

$$D = K\lambda / \beta \cos\theta$$

Where,

K is a dimensionlessshape factor, with a value close to unity.

The shape factor has a typical value of about 0.9, but varies with the actual shape of the crystallite;

λ is the X-ray wavelength;

β is the line broadening at half the maximum intensity (FWHM), after subtracting the instrumental line broadening, in radians. This quantity is also sometimes denoted as θ is the Bragg angle.The 2θ value of 23.46°, 27.07°, 31.78°, 34.53°, 37.40°, 41.03° indicate the crystalline nature of prepared sulphur nanoparticles and the crystalline size was determined to be 23.4nm [27-29].

3.5. SEM with EDS Analysis

The structural characteristics of biosynthesized SNPs were studied by scanning electron microscopy The SEM specimen was prepared by first drop-coating SNFs on a mica film followed by sputter coating of gold, then the mica film was transferred on to a sample holder made of carbon. Fig. 7a & 7b shows SEM images of SNPs. It is obvious that the particles had a low degree of agglomeration.

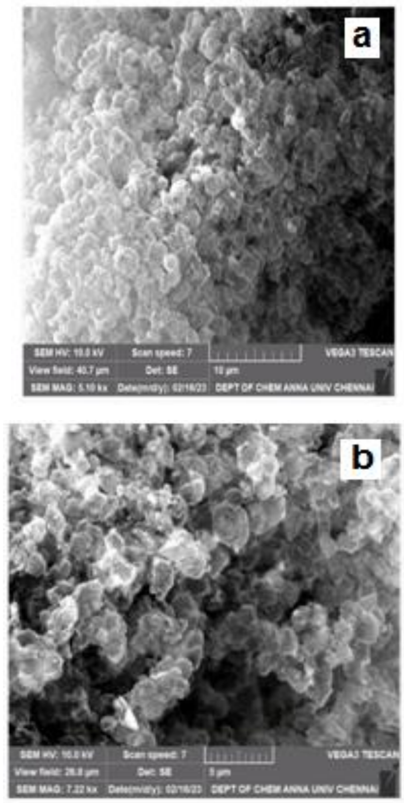

Figure 7. SEM images of SNPs

The SEM micrographs depicted that the particle size ranged from ~25 to ~120 nm. The elemental composition of SNPs was identified by EDS. The energy dispersive X-ray spectroscopy **spectrum (Fig. 8)** showed the presence of sulphur. Other elements such as Na, O and C were also observed due mainly to the presence of the by-product, sodium citrate.

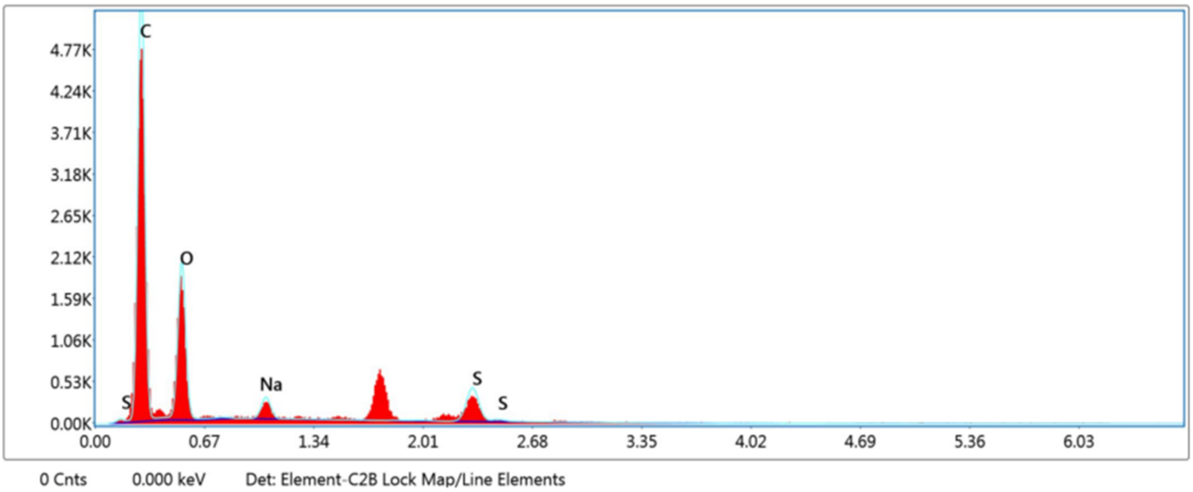

Figure 8. Energy Dispersive X-ray spectroscopy spectrum of Biosynthesized sulphur nanoparticles

3.6.Antibacterial activity of SNPs

The inhibitory effect of prepared sulphur nanoparticles on common pathogenic bacteria, E. coli, and S. aureus, was assessed through disc diffusion and minimum inhibitory concentration methods. The zone of inhibition values obtained isshown in Fig. 9. Once the medium solidified, bacterial inoculums were evenly spread on the plates using a sterile swab moistened with the bacterial suspension. The antibacterial study results revealed significantly heightened bioactivity of sulphur nanoparticles synthesized from F. religiosa leaves against both pathogens, as indicated in Table. 1 [27].

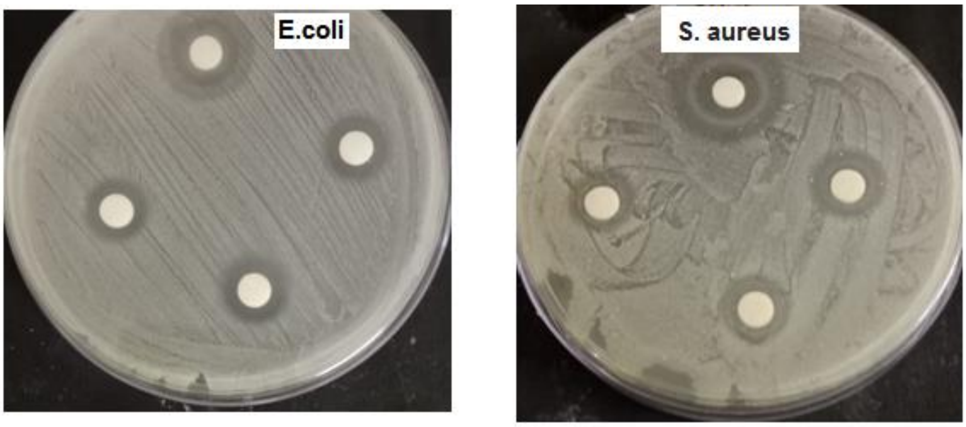

Figure 9. Antibacterial study of sulphur nanoparticles prepared from F.religiosa leaves

Table 1. Antibacterial activity of SNPs

	Zone of inhibition (mm) Sample (µg/ml)				
ORganisms	1000	750	500	Standard	SD
Staphylococcus aureus	18	18	18	20	1
Escherichia coli	14	14	14	20	3

4. CONCLUSIONS

A cost effective, rapid, environmentally friendly method was used for the synthesis of sulphur nano particles using *F.religiosa* leaf extract.The nanoparticles were characterized using UV-Vis, FTIR, XRD, FESEM, the synthesized sulphur nanoparticles were confirmed to be in nano meter scale with the help of X-ray diffraction method.

The SEM analysis have revealed that the synthesized sulphur nano particles have a spherical size and from XRD the crystalline size of the sulphur nano particles is found to be 23.4 nm

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IZVOD

BIO-INSPIRISANA SINTEZA NANOČESTICA SUMPORAI NJEGOVA PRIMENA

Koriscen je ekonomičan, brz i ekološki prihvatljiv pristup za sintezu nanočestica sumpora, koristeći ekstrakt iz listova Ficus religiosa. Sveobuhvatna karakterizacija ovih nanočestica obavljena je UV-vidljivom spektroskopijom, FTIR, XRD i FESEM tehnikama. Metoda difrakcije rendgenskih zraka nedvosmisleno je potvrdila nanometarske dimenzije sintetizovanih nanočestica sumpora. SEM analiza je razjasnila njihovu sfernu morfologiju, dok XRD podaci ukazuju na veličinu kristala od 23,4 nm za ove pripremljene nanočestice. Pored toga, antibakterijska procena nanočestica sumpora proizvedenih iz listova Ficus religiosa pokazala je superiornu bioaktivnost protiv štetnih vrsta bakterija kao što su Escherichia Coli i Staphilococcus aureus.

Ključne reči: *nanočestice sumpora, Ficus religiosa, bioaktivnost, zelena sinteza*

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Jeyaprabha Chellappa : https://orcid.org/0000-0001-6729-2157
Sribharathy Vijayagopal: https://orcid.org/0000-0002-0542-3569